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(71) Applicant: **SOCIETE DES PRODUITS NESTLE S.A.**
1800 Vevey (CH)

(72) Inventors:
- **Elli, Maria**
  1010 Lausane (CH)
- **Zink, Ralf**
  1801 Le Mont Pelerin (CH)
- **Marchesini-Huber, Barbara**
  1073 Savigny (CH)
- **Reniero, Roberto**
  1801 Le Mont Pelerin (CH)

(74) Representative: **Becker Kurig Straus**
Patentanwälte
Bavariastrasse 7
80336 München (DE)

(54) Synthetic medium for cultivating lactobacillus and/or bifidobacteria

(57) The present invention relates to a novel defined medium suitable to cultivate lactic acid bacteria, such as *Lactobacillus* or *Bifidobacteria*. In particular the present invention pertains to the use of said medium for the isolation of bioactive molecules or functional metabolites.

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Description

[0001] The present invention relates to a novel synthetic medium suitable to cultivate lactic acid bacteria of the genus *Bifidobacteria* or *Lactobacillus* which contains particular nucleotides and deoxynucleotides. In particular, the present invention pertains to the use of said medium for the isolation of bioactive molecules or functional metabolites.

[0002] Lactobacilli are widely distributed in nature and are largely used for industrial fermentation processes for e. g. the preparation of dairy products. In recent years the study of their metabolism has been greatly enhanced since particular strains have been found to exert a positive effect on the maintenance of the healthy state of host organisms. Their complex nutrient requirements are usually satisfied by natural sources or synthetic growth media, containing matrices of undefined and complex composition, such as yeast extract and peptones of various origins.

[0003] Some semi-synthetic and completely chemically defined media have been developed for lactic acid bacteria for different purposes, such as the investigation of the nutritional requirements of bacterial cells, the identification of the role of specific components by detection of the effects after removal thereof from the medium or the isolation of mutants auxotrophic for certain substances. Growth media with a defined chemical composition were also used to determine the requirements of Lactobacilli for nucleotides and to attribute their essential or non-essential role with regard to different DNA precursors.

[0004] In the past few decades several studies were performed by means of defined media, on the strain *Lactobacillus johnsonii* ATCC 11506 (formerly known as *Lactobacillus acidophilus* R-26), firstly proposed by Hoff-Jorgensen as an experimental organism for determining the presence of DNA residues in biological samples (Hoff-Jorgensen, "A microbiological assay for deoxyribonucleosides and deoxyribonucleic acid", Biochem J. **50** (1952), 400-403). Ives and Ikeda report in "Life on the salvage path: the deoxynucleoside kinases of *Lactobacillus acidophilus* R26", Progr. Nucl. Acid. Res. (1998), 207-252, that this strain requires the presence of at least one deoxyribonucleoside in the growth medium due to the functional absence of ribonucleotide reductase activity.

[0005] Further, it could be shown that in *Lactobacillus delbrueckii subsp. lactis* ATCC 7830 (formerly known as *L. leichmannii* ATCC 7830), in contrast to strain R-26, the requirement for deoxyribonucleosides could be replaced by Vitamin B12.

[0006] The latter strain was subjected to several investigations in order to elucidate the nucleotide requirements of Lactobacilli and the effects of supplementation of the medium with DNA molecules (Jeener & Jeener, Exptl. Cell Res. **3** (1952), 675-680; Okazaki & Okazaki, J. Biochem. **35** (1959), 434-445; Hoff-Jorgensen, Meth. Enzymol. **3** (1957), 781-785; Mc Nutt, Meth. Enzymol. **2** (1955), 464-468; Lovtrup & Shugar, J. Bacteriol. **82** (1961), 623-631.

[0007] Thymidine was often indicated as a key factor for the growth of *Lactobacillus acidophilus'and L. leichmannii.* Further, in later studies the removal of uracil was demonstrated to deeply affect RNA synthesis and cell division in lactic acid bacteria.

[0008] In J. Bacteriol. **73** (1957), 670-675 Siedler et al reported an optimization of the Hoff-Jorgensen's medium by studying the ability of uracil, vitamin B6 and acid-hydrolized casein to reproduce the positive effect of yeast extract on *L. acidophilus* development in a semi-defined medium.

[0009] Recently, Imbert & Blondeau disclosed in Curr. Microbiol. **37** (1998), 64-66, a chemically defined medium to examine the ability of some *Lactobacillus* species to grow after iron chelation and furthermore the interaction between manganese and iron was examined. The supplementation of chelated iron did not affect bacterial growth in the presence of manganese, while a slightly positive effect was observed following to the addition thereof to the same medium deprived of manganese especially for *L. acidophilus* ATCC 4356T after aerobic incubation.

[0010] It is known that most pathogenic bacteria require iron for their growth. In contrast thereto, lactic acid bacteria have been generally recognized as exceptions among the living organisms in that they do not show such an indispensable iron requirement which is therefore considered to represent an ecological advantage against pathogens in natural environments.

[0011] Merely few publications exist reporting the average content of metal in lactic acid bacteria. In general, a strong variability among the *Lactobacillus* species has been found exemplified by a comparison between the iron content of *Lactobacillus plantarum* and *Escherichia coli* cells in which a lower level in the former species was confirmed (Archibald et al. in FEMS Microbiol. Lett. **19** (1983), 29-32).

[0012] Recently, particular strains of the genus *Lactobacillus* and *Bifidobacteria* have attracted great attention since properties beneficial to the host organism have been attributed to them. So far it is merely known that these strains show the properties reported, yet the reason for this property has not been elucidated.

[0013] In this respect EP 0 577 903 discloses the use of lactic acid bacteria, especially a *Lactobacillus* strain which upon ingestion reveals beneficial effects to organisms infested by *Helicobacter pylori*. Accordingly, the *Lactobacillus* is obviously capable to produce metabolic compounds capable to prevent a further growth and/or adhesion of *Helicobacter* to gastric and/or intestinal mucosal structures. From the point of view of determining said metabolic compound it would be desirable to have a medium from which compounds produced by the lactic acid bacteria may be isolated.

[0014] In order to isolate said compounds the bacterial cells shall be cultivated to a reasonable extent in the medium.

Yet, media providing a sufficient growth of lactic acid bacteria are normally not defined and comprise complex matrices, such as yeast extract and peptones, from which a desired, still unknown compound cannot be isolated.

[0015] On the other hand, defined media so far known are normally specific for a given bacterial strain and moreover do not provide for a sufficient growth of the microorganism.

[0016] Consequently, a problem of the present invention is to provide a novel defined medium, which allows for a sufficient growth of a plurality of different bacterial strains.

[0017] This problem has been solved by providing a synthetic medium for cultivating lactic acid bacteria belonging to the genus *Lactobacillus* or *Bifidobacteria* comprising a carbon source, buffer, a nitrogen source, trace elements, antioxidants and vitamins characterized in that it contains two free bases, one ribonucleoside and two 2'-deoxynucleosides, each in an amount sufficient to promote growth of the microorganisms.

[0018] During the extensive studies leading to the present invention, a chemically defined growth medium for *Lactobacillus johnsonii* was developed, which' was surprisingly found to be suitable for the cultivation of other Lactobacilli and/or *Bifidobacteria* as well. In the experiments particular attention has been paid to the nucleotide composition of the medium and several sources of DNA precursors were examined for their ability to support *Lactobacillus/Bifidobacteria* growth.

[0019] To this end a defined medium for *L. johnsonii* was supplemented with free bases (adenine, cytosine, guanine, thymine, uracil and inosine), ribonucleosides (adenosine, cytidine, guanosine, uridine) and deoxyribonucleosides (2'-deoxyadenosine, 2'-deoxycytidine, 2'-deoxyguanosine, 2'-deoxyuridine and thymidine). The different investigated Lactobacilli showed the ability to grow in the defined medium in the simultaneous presence of all the five free bases, all four ribonucleosides and all the five deoxyribonucleosides, whereby the minimal requirement for substantial growth has been found to be a combination of at least two free bases, one nucleoside and two deoxyribonucleosides.

[0020] It could be shown that both adenine and guanine could be replaced by inosine as precursor and the requirement for thymine and cytosine could be satisfied by supplementation of the medium with uracil. The presence of inosine and uracil was found to be beneficial for the growth of some *Lactobacillus* species, confirming their inability to substantially synthesize purines and pyrimidines *de-novo*.

[0021] The supplementation of the defined medium with the above minimal required compounds was able to increase the final cell counts. However, optimal results were obtained with a combination of the following nucleotide derivatives: guanine, thymine, cytidine, deoxyadenosine and deoxyuridine.

[0022] This particular recipe was also used to investigate the iron requirements of Lactobacilli by means of several defined recipes differing in their nucleotide composition. Little differences in the optical density values were observed after 18 hours incubation at 37°C after removal of the iron compound when the minimal number of the required nucleotide precursors were supplied.

[0023] Stronger effects of iron removal were detected if inosine and uracil were supplied as the only nucleotide sources. Further investigations allowed to show that the negative effect of iron omission was emphasized after uracil replacement with cytosine. Therefore, a putative role of iron in the metabolism of pyrimidines or purines of Lactobacilli/Bifidobacterium was proposed. It is concluded that *Lactobacillus spp., particularly L. johnsonii,* require iron only under particular environmental conditions. Yet, when supplementing a synthetic medium with at least two free bases, one ribonucleoside and two deoxyribonucleotides as nucleotide precursors a substantial growth of different Lactobacilli and Bifidobacteria could be shown, without the need to add iron to the medium. This feature proves to be rather advantageous due to the fact that contamination of the culture by bacteria requiring iron for their growth may be excluded thereby.

[0024] As the carbon source for the medium any source well known in the art, e.g. fructose, lactose, saccharose or mixtures thereof, may be selected. In order to provide a pH-value adapted to the specificity of the particular strains the medium may contain any sort of buffer used in the art, such as KH_2PO_4/K_2HPO_4, diammoniumhydrogencitrate, $NaHCO_3/Na_2CO_3$ or mixtures thereof.

[0025] The medium further contains a nitrogen source which may preferably be selected from any of the natural amino acids or diammoniumhydrogencitrate or mixtures thereof.

[0026] So as to provide a suitable environment for growth the medium further contains antioxidants. Antioxidants are well known in the art such as e.g. ascorbic acid, cysteine, thiol compounds or mixtures thereof. For the purpose of reducing the number of different compounds included in the synthetic medium cysteine is preferred as such an antioxidant.

[0027] Further, the medium contains trace elements required for the growth of the microorganisms. Said trace elements are e.g. Cu-, Zn-, Mn-, Mg-, Co-compounds, or mixtures thereof. For the purpose of reducing the amount of compounds in the medium the counterion shall preferably be selected from another organic compound to be added to the medium, such as e.g. citrate, or may be a negatively charged ion , such as Cl^- etc..

[0028] The medium additionally contains different vitamins, such as nicotinic acid, panthotenate, cobalamine, p-aminobenzoic acid, pyridoxal-HCl, riboflavin, biotin, folic acid or mixtures thereof.

[0029] It will be appreciated that the skilled person will, based on his own knowledge, use compounds not explicitly

listed above, yet providing for the same purpose.

[0030] It has been found that a preferred amount for the nucleotide precursors to be included in the medium ranges from about 0.5 g to about 0.3 g /l, preferably about 0.1 g /l.

[0031] Due to its defined composition the present medium may be used for the identification and/or isolation of bioactive molecules and/or functional metabolites, respectively, produced by Lactobacilli and/or *Bifidobacteria*. In this respect the bacteria are grown in the medium. Since this medium provides for a suitable growth environment a high cell count may be achieved with the result that also a substantial amount of bioactive molecules/functional metabolites may be produced.

[0032] For the isolation of metabolic compounds secreted by the microorganism the defined cultivation medium may be centrifuged at high speed so as to deplete it from any bacterial cells. The supernatant may then be collected and further analyzed for biological compounds according to techniques well known in the art.

[0033] The invention will now be described by means of examples which are not construed to limit the same.

Examples

Bacterial strains:

[0034] For the experiments the following different strains were used:

Table 1:

Origin of investigated bacterial strains	
L.johnsonii ATCC 33200^T	American Type Culture Collection
L. johnsonii ATCC 11506	American Type Culture Collection
L. johnsonii Lal (NCC 533)	Nestec Culture Collection
L. johnsonii ATCC 332	Deutsche Sammlung für Mikroorganismen
L. johnsonii DSM 20553	Deutsche Sammlung für Mikroorganismen
L. gasseri DSM 20243^T	Deutsche Sammlung für Mikroorganismen
L. gallinarum DSM 33199^T	Deutsche Sammlung far Mikroorganismen
L.casei ATCC 393^T	American Type Culture Collection
L. paracasei NCDO 151^T	National Collection of Dairy Organism (now NCFB)
L. plantarum NCDO 1193	National Collection of Dairy Organism (now NCFB)
L. helveticus ATCC 10386	American Type Culture Collection
L. delbrueckii subsp. *delbrueckii* DSM 20074^T	Deutsche Sammlung far Mikroorganismen
L. delbrueckii subsp. *lactis* ATCC 7830	American Type Culture Collection

[0035] The microorganisms were propagated in MRS (Difco) broth or agar at 37°C. Two subculturing steps of 18 hours each were performed from a frozen culture prior to performing the tests.

Media:

[0036] The composition of a defined medium (**DM1**) is indicated in **Table 2**, below:

Table 2:

Medium composition of the defined medium	
Constituent	**Final concentration (g/l)**
Glucose	10
Potassium hydrogen phosphate	3.1
di-ammonium hydrogen citrate	2
Potassium dihydrogen phosphate	1.5

Table 2: (continued)

Medium composition of the defined medium	
Constituent	**Final concentration (g/l)**
Sodium chloride	0.02
Ascorbic acid	0.5
Potassium acetate	10
Tween 80	1
Heptahydrated magnesium sulphate	0.5
Hydrated manganese sulphate	0.02
Cobalt sulphate	0.5
Calcium lactate	1
DL-alanine	0.2
DL-aminobutyric acid	0.1
Glycine	0.2
L-histidine HCl	0.2
L-lysine HCl	0.2
L-phenylalanine	0.1
L-proline	0.2
L-serine	0.1
L-threonine	0.1
L-cysteine	0.1
L-arginine	0.2
L-aspartic acid	0.3
L-asparagine	0.1
L-glutamic acid	0.3
L-isoleucine	0.1
L-leucine	0.2
L-methionine	0.1
L-tyrosine	0.1
L-tryptophan	0.1
L-valine	0.1
Nicotinic acid	10 mg
Calcium pantothenate	10 mg
Cyanocobalamin	0.02 mg
Para-aminobenzoic acid	0.2 mg
Myo-inositol	10 mg
Pyridoxal HCl	10 mg
Riboflavin	10 mg
Biotin	1 mg
Folic acid	0.2 mg

Table 2: (continued)

Medium composition of the defined medium	
Constituent	**Final concentration (g/l)**
Guanine	0.1
Thymine	0.1
Cytidine	0.1
2'-deoxyadenosine	0.1
2'-deoxyuridine	0.1

[0037] The above recipe lacks iron that was supplemented as ferrous sulphate ($FeSO4\text{-}7H_2O$) (0.02 g/l final concentration) dissolved in sterile distilled water, freshly prepared each time and immediately added to the medium. It was filter-sterilized or autoclaved (121°C). Each listed component was supplied by Sigma Chemicals. The nucleotides indicated in the recipe represent the optimal combination capable to support *L. johnsonii* growth at a high level.

[0038] Other nucleotide derivatives were tested:

Free bases : adenine, guanine, cytosine, uracil, thymine;
ribonucleosides : adenosine, cytidine, guanosine, uridine; and
deoxyribonucleosides: 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, 2'-deoxyuridine, thymidine,

that were supplied as neutral or alkaline solutions with the same final concentration indicated in **Table 2.**

Viable cells and optical density measurement:

[0039] The number of viable cells was determined by decimal counts after anaerobic incubation for 48 hours on agar MRS (Difco) plates at 37°C. The optical density was measured at 560 nm using a Dye UNICAM PU8660 spectrophotometer. The reported growth results raise from the average of three trials.

Inoculum preparation:

[0040] The tested defined media were 1% inoculated from an MRS culture, washed twice and finally resuspended with the same amount of sterile distilled water in order to avoid nutrient transfer via the medium.

Incubation parameters:

[0041] The tubes were incubated at 37°C for 18 hours.

[0042] The defined medium as above was optimized in composition for *L. johnsonii* for the ability to achieve high growth level. After 18 hours incubation at 37°C, 1.8 logs in average were gained for all the strains listed above.

[0043] As may be derived from **Table 2**, the medium contains a combination of different DNA derivatives (two free bases, one ribonucleoside and two 2'-deoxyribonucleosides). Another mix of nucleotides was tested which presented inosine as purine precursor and uracil as the only essential pyrimidine base. The modified medium supported the growth of the strains in a range of 1.5-2 logs increasing after 18 hours incubation.

[0044] The omission of all the DNA and RNA precursors resulted in an almost complete growth inhibition of nearly all of the tested species with the proviso of *L. casei subsp. casei, L.casei subsp. paracasei* and *L. plantarum* which were not affected by this depletion, confirming that they can synthesize purines and pyrimidines by *"de-novo"* synthesis, allowing the build up of the nucleotide ring directly on the activated ribose molecule.

[0045] The other tested strains required at least inosine and uracil to synthesize the nucleotides pool essential for RNA and DNA synthesis. The omission of iron did not affect the ability of the tested strain to grow in this depleted medium as may be seen from **Table 3** below:

Table 3:

Effect of iron removal from the defined medium DM1			
Strain		Final growth yield(a)	
		DM1	DM1 (b)
L. johnsonii	ATCC 33200^T	1.65	1.75
L. johnsonii	ATCC 11506	0.67	0.65
L. johnsonii	Lal (NCC 533)	1.71	1.76
L. johnsonii	DSM 20553	1.10	1.20
L. gasseri	DSM 20243^T	1.50	1.41
L. gallinarum	DSM 33199^T	1.97	2.00
L. casei	ATCC 393^T	1.95	1.93
L. paracasei	NCDO 151^T	1.12	1.30
L. delbrueckii subsp. *delbrueckii*	DSM 20074^T	1.40	1.52
L. plantarum	NCDO 1193	1.30	1.35
L. delbrueckii subsp. *lactis*	ATCC 7830	1.90	1.94

(a) Results expressed as O.D. at 560 nm

(b) DM1 recipe deprived of ferrous sulphate

[0046] Different nucleotide sources were added to the medium to replace the **DM1** nucleotide composition. Five free bases (adenine, cytosine, guanine, thymine and uracil), four ribonucleosides (adenosine, cytidine, guanosine and uridine) or five 2'-deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine, thymidine) were supplied and the corresponding media were termed **DM3, DM4** and **DM5,** respectively (**Table 4**).

Table 4

Effect of free bases, ribonucleosides and deoxyribonucleosides on Lactobacilli growth and performance after iron depletion						
Strain	Final growth yield (a)					
	DM3	DM3 (b)	DM4	DM4 (b)	DM5	DM5 (b)
L. johnsonii ATCC 33200 T	1.00	0.97	1.13	1.10	0.92	0.48
L. johnsonii Lal (NCC 533)	0.78	0.75	1.16	0.86	1.14	0.95
L. johnsonii ATCC 11506	0.68	0.69	0.67	0.56	0.93	0.94
L. johnsonii DSM 20553	0.78	0.74	1.04	0.85	1.07	1.04
L. gasseri DSM 20243 T	0.92	0.95	0.92	0.87	0.88	0.86
L. gallinarum ATCC 33199 T	0.41	0.37	0.78	0.71	1.05	0.93
L. casei ATCC 393 T	0.91	0.87	1.24	1.23	1.21	1.22
L. paracasei NCDO 151 T	1.00	1.02	0.98	0.94	1.03	1.08
L. delbrueckii DSM 20074 T	0.15	0.15	0.34	0.29	0.26	0.25
L. lactis ATCC 7830	0.84	1.03	1.12	1.12	0.96	1.11
L. *helveticus* ATCC 892	0.04	0.04	0.11	0.07	0.05	0.06

(a) results expressed as O.D. at 560 nm

(b) omission of ferrous sulphate

[0047] The performance of the tested strains towards the different DNA derivatives was determined by measuring the O.D. values at 560 nm. **Table 5** shows the final growth yield achieved both when the strains were grown in the

modified media and when iron, in form of ferrous sulphate, was omitted. The results showed no strong effects of this removal according to the absence of a clear iron requirement of Lactobacilli.

Table 5

Effect of ferrous sulphate depletion from the medium **DM2** on Lactobacilli growth		
Strain	**Final growth yield (a)**	**Final growth yield (a)**
	DM2	**DM2 (b)**
L. johnsonii ATCC 33200 T	0.82	0.46
L. johnsonii Lal (NCC 533)	1.13	0.67
L. johnsonii ATCC 11506	0.57	0.15
L. johnsonii ATCC 332	1.10	0.65
L. johnsonii DSM 20553	1.20	0.36
L. gasseri DSM 20243 T	0.82	0.57
L. gallinarum ATCC 33199 T	0.82	0.50
L. casei ATCC 393 T	1.24	1.23
L. paracasei NCDO 151 T	1.16	1.02
L. delbrueckii DSM 20074 T	0.85	0.52
L. plantarum NCDO 1193	1.38	1.38
L. lactis ATCC 7830	1.20	1.14
L. helveticus ATCC 892	1.13	0.84

(a) results expressed as O.D. at 560 nm

(b) Depletion of ferrous sulphate

[0048] As expected *L. johnsonii* ATCC 11506 developed rather poorly in this medium but the effect of iron removal was slightly observable.

[0049] The iron deprivation was applied also to the **DM2** medium characterized by the presence of inosine and uracil as nucleotide sources (**Table 4 and 6**). In this case a stronger effect was observed especially for L. *johnsonii*, *L. gasseri*, *L. gallinarum* and *L. helveticus* which showed a significant decrease in the optical density values after incubation in the **DM2** medium deprived of ferrous sulphate.

Table 6

Effect of adenine, guanine, inosine, uracil, cytosine and orotic acid on *L. johnsonii* growth in the defined medium DM1 with and without ferrous sulphate addition					
Medium	***L. johnsonii*** **strains**				
	ATCC 33200 T	**La1 (NCC 533)**	**ATCC 11506**	**ATCC 332**	**DSM 20553**
(A) + $FeSO_4$	0.72	1.14	0.57	0.97	1.11
(A) - $FeSO_4$	0.48	0.19	0.21	0.41	0.41
(B) + $FeSO_4$	0.86	1.05	0.58	0.23	1.15
(B) - $FeSO_4$	0.12	0.13	0.16	0.10	0.16
(C) + $FeSO_4$	0.43	1.07	0.52	0.77	1.06
(C) - $FeSO_4$	0.51	0.17	0.21	0.52	0.39
(D) + $FeSO_4$	0.25	0.23	0.31	0.24	0.26
(D) - $FeSO_4$	0.16	0.12	0.18	0.15	0.21
(E) + $FeSO_4$	0.36	0.34	0.31	0.47	0.35

Table 6 (continued)

Effect of adenine, guanine, inosine, uracil, cytosine and orotic acid on *L. johnsonii* growth in the defined medium DM1 with and without ferrous sulphate addition					
Medium	***L. johnsonii* strains**				
	ATCC 33200 T	**La1 (NCC 533)**	**ATCC 11506**	**ATCC 332**	**DSM 20553**
(E) - $FeSO_4$	0.40	0.25	0.30	0.50	0.48
(F) + $FeSO_4$	0.44	0.39	0.49	0.62	0.89
(F) - $FeSO_4$	0.43	0.30	0.41	0.25	0.38
(G) + $FeSO_4$	0.33	0.49	0.49	0.67	0.89
(G) - $FeSO_4$	0.40	0.29	0.33	0.32	0.47
(H) + $FeSO_4$	0.31	0.28	0.43	0.25	1.06
(H) - $FeSO_4$	0.21	0.22	0.32	0.24	0.23
(A) DM1 + inosine, uracil = **DM2** (B) DM1 + inosine, cytosine; (C) DM1 + inosine, uracil, cytosine; (D) DM1 + cytosine; (E) DM1 + inosine, orotic acid; (F) DM1 + adenine, guanine, orotic acid; (G) DM1 + adenine, guanine, uracil; (H) DM1 + adenine, guanine, cytosine.					

Claims

1. Synthetic medium for cultivating lactic acid bacteria belonging to the genus *Lactobacillus* or *Bifidobacteria* comprising a carbon source, buffer, a nitrogen scource, trace elements, antioxidants and vitamins characterized in that it contains at least two free bases, one ribonucleoside and two 2'-deoxynucleosides, each in an amount sufficient to promote growth of the microorganisms.

2. The medium according to claim 1, wherein the carbon source is selected from the group consisting of glucose, fructose, lactose, saccharose or a mixture thereof.

3. The medium according to any of the claims 1 and 2, wherein the buffer is selected from the group consisting of KH_2PO_4/K_2HPO_4, diammoniumhydrogencitrate, $NaHCO_3/Na_2CO_3$ or a mixture thereof.

4. The medium according to any of the preceding claims, wherein the nitrogen source is selected from one or more amino acids, diammoniumhydrogencitrate or any mixture thereof.

5. The medium according to any of the preceding claims, wherein the antioxidant is selected from ascorbic acid, cysteine, thiol compounds or any mixture thereof.

6. The medium according to any of the preceding claims, wherein the trace elements are selected from Cu-, Zn-, Mn-, Mg-, Co-compounds or any mixture thereof.

7. The medium according to any of the preceding claims, wherein the vitamins are selected from the group consisting of nicotinic acid, panthotenate, cobalamine, p-aminobenzoic acid, pyridoxal-HCl, riboflavin, biotin, folic acid or any mixture thereof.

8. The medium according to any of the preceding claims wherein the nucleotides and deoxynuclotides are included in an amount of from about 0.3 g to about 0.5 g /l, preferably about 0.1 g /l.

9. Use of a medium according to any of the preceding claims for the growth of lactic acid bacteria belonging to the genus *Lactobacillus* and/or *Bifidobacteria*.

10. Use of a medium according to any of the preceding claims for the identification and/or isolation of bioactive molecules or functional metabolites.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 10 5855

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION
X	EP 0 460 414 A (KAJANDER E OLAVI) 11 December 1991 (1991-12-11) * page 5, line 30,41; page 6, line 8-16	1-8,10	C12N1/20
X	PATENT ABSTRACTS OF JAPAN vol. 007, no. 122 (C-168), 26 May 1983 (1983-05-26) & JP 58 040096 A (NIPPON ZEON KK), 8 March 1983 (1983-03-08) * abstract *	1-8,10	
A	EP 0 656 421 A (BIO MERIEUX) 7 June 1995 (1995-06-07) * claim 1 *		
A	PATENT ABSTRACTS OF JAPAN vol. 015, no. 337 (C-0862), 27 August 1991 (1991-08-27) & JP 03 130071 A (KIRIN BREWERY CO LTD), 3 June 1991 (1991-06-03) * abstract *		TECHNICAL FIELDS SEARCHED C12N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23 August 1999	Lonnoy, O

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